Compromised Bond Strength after Root Dentin Deproteinization Reversed with Ascorbic Acid

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Abstract

Introduction: The present study evaluated the effect of a reducing agent on the bond strength of deproteinized root canal dentin surfaces when using a self-adhesive versus dual-cured cement. Regional differences were also evaluated. **Methods:** A total of 45 bovine incisor roots were divided into 3 groups: irrigation with physiologic solution (control), 10-minute deproteinization with 5% NaOCl, and 10-minute deproteinization with 5% NaOCl followed by 10 minutes of 10% ascorbic acid. Fiber posts were cemented with either RelyX U100 or RelyX ARC (with SingleBond 2 or Clearfil SE Bond). The push-out bond strength was evaluated after 24 hours of storage. Data were submitted to three-way analyses of variance and Dunnett T3 tests (α = 0.05). **Results:** No differences between cements were observed within the testing conditions, regardless of the adhesive (P < .05). Deproteinization reduced bond strengths. Subsequent treatment with ascorbic acid was capable of reversing bond strength value changes to levels similar to those of controls. Regional radicular differences were also found, where coronal > middle > apical. **Conclusions:** The reducing agent was capable of reversing the effect of dentin deproteinization, and RelyX U100 behaved similarly to RelyX ARC. *(J Endod 2010;36:130–134)*

Key Words

Ascorbic acid, dental materials, dentin, shear strength, sodium hypochlorite

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Endodontically treated teeth frequently have little remaining structure and require intraradicular retention before restoration (1, 2). Metallic posts have been traditionally used for intraradicular retention. However, these materials have a high elastic modulus and, therefore, are more likely to cause fracture of the remaining dental structure (3). This problem spurred further research and the development of fiber-reinforced posts as more flexible materials, with an elastic modulus similar to that of dentin (4).

Fiber posts are normally used along with resin-based cements. These cements have been increasingly accepted, especially when their physical-chemical properties are considered (5). However, the luting of a fiber post into the root canal dentin is a step that remains controversial. Several factors might influence the retention of fiber posts. Among these variables are the application method, type of adhesive, surface treatment of the post, type of cement, and irrigation protocol (6–16). Because bonding is mainly a micromechanical retention–mediated interaction between the demineralized dentin and resin tag infiltration (17), the irrigation protocol is an important clinical step; this is particularly true when the variations in the extent of smear layer removal by various solutions are considered (18). In addition, the adhesion to root dentin is also influenced by the concentration and direction of dentin tubules at different levels of the root canal walls and the ease of access to the radicular thirds of the root (19–22).

Recently, the introduction of self-adhesive cements has given a new perspective for post cementation (23). Self-adhesive cements are new, and information regarding their characteristics or clinical effectiveness remains restricted (24, 25). According to one manufacturer, the acidic methacrylate monomers present in the cement contain phosphoric acid groups that are highly reactive as a result of their high number of carbon double-bonds. The phosphoric acid groups contribute to the self-adhesion and are able to react with fillers in a neutralization reaction. During this neutralization reaction, fluoride ions are released from the fillers. The manufacturer also recommends the pretreatment of dentin with sodium hypochlorite (NaOCl) at concentrations of 2.5%–5.25% before post cementation (26).

It has been previously established that treatment with NaOCl is an effective dentin deproteinization method (27, 28). This procedure has also been proposed as a method to improve adhesive wettability (29). However, the bonding performance on deproteinized dentin differs between adhesives (27, 30, 31). Therefore, it is crucial to compare new self-adhesive cements with conventional ones by using different adhesives.

Although it is an effective protein denaturant, NaOCl is also a potent biologic oxidant. Therefore, biocompatible reducing agents (eg, ascorbic acid and sodium ascorbate) have been studied to reverse the negative effects of oxidants (16, 32–34). Ascorbic acid neutralizes residual NaOCl via a redox reaction (35). Thus, the aims of the present study were (1) to determine the shear bond strength of 2 luting cements on NaOCl-deproteinized root dentin surfaces and (2) to evaluate the ability of 10% ascorbic acid to reverse possible negative effects of NaOCl. If reduced bond strength occurs on NaOCl-treated dentin as a result of the oxidizing action of NaOCl, we hypothesized that the compromised bond strength will be reversed by the reduction before resin bonding occurs. We also hypothesized that there might be differences between cements as well as regional areas in the cervical, middle, and apical thirds of the root.

Materials and Methods

The cements and adhesives studied are presented in Table 1. Forty-five freshly extracted bovine incisors with anatomically similar root segments and fully developed

TABLE 1. Materials Used in the Study, along with the Manufacturer's Instructions

Material	Manufacturer	Batch	Composition	Instructions
Rely X U100 Self-Adhesive Universal Resin Cement	3 M ESPE, Seefeld, Germany	339395	Glass fillers, silica, calcium hydroxide, self-cure initiators, pigments, light-cure initiators; methacryled phosphoric esters, dimethacrylates, acetate, stabilizers, self-cure initiators	Rinse with water and dry with paper points; mix the 2 pastes into a homogenous paste within 20 seconds (s); light-curing = 20 s
Rely X ARC Adhesive Resin Universal Cement Paste	3 M ESPE, St Paul MN	GHHM	Bis-phenol A diglycidymethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), zirconia/ silica filler 68%, proprietary dimethacrylate monomer, amine	Mix the 2 pastes into a homogenous paste within 10 s; light-curing = 40 s
Adper Single Bond 2	3 M ESPE, St Paul MN	51202	Bis-GMA, 2-hydroxyethyl methacrylate (HEMA), polyalkenoic, acid copolymer, photoinitiators, ethanol, water	Phosphoric acid-etching for 15 s; rinse with water within 10 s; dry with paper points; 2 coats of adhesive were applied; air-dried for 5 s; light-curing = 10 s
Clearfil SE Bond	Kuraray Medical Inc, Tokyo, Japan	51429	Primer: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), HEMA, hydrophilic dimethacrylate, di-camphorquinone, aromatic tert-amine, water. Bonding: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, photo initiator, aromatic tert-amine, silanated colloidal sillica	Primer was applied 20 s; air-dried for 5 s; bond was applied; air-dried for 5 s; light-curing = 10 s

apices were selected and cleaned in an ultrasonic water bath for 10 minutes (Ultrasonic Cleaner 1440D; Odontobrás Ltda, Ribeirao, SP, Brazil). The roots were cut to a uniform length of 17 mm from the apical end.

The same operator instrumented and obturated with gutta-percha and a calcium hydroxide sealer (Sealer 26; Dentsply, Sao Paulo, SP, Brazil) all root canals. A CaOH-based sealer was selected to avoid adverse effects from eugenol-based sealers (6). Root canal openings were filled with a glass ionomer cement (Vidrion R; SSWhite, Rio de Janeiro, RJ, Brazil), and the samples were stored at 37°C in distilled water for 1 week. After this period, the post spaces were all prepared to a depth of 13 mm with special preparation drills supplied by the manufacturer of the fiber posts (Exacto #3; Angelus, Londrina, SP, Brazil). Posts used were nontransilluminating posts tapered with a coronal diameter of 2.0 mm and an apical diameter of 1.1 mm. Roots were rinsed with water to remove remaining debris, dried with paper points, and then divided into 3 groups (n = 15): group 1, roots were irrigated with physiologic saline solution for 10 minutes (control); group 2, roots were irrigated with 5.25v/v% NaOCl for 10 minutes; and group 3, roots were irrigated with 5.25v/v% NaOCl for 10 minutes, rinsed with water, and rinsed with freshly prepared 10% ascorbic acid for 10 minutes. The 10-minute irrigation with NaOCl was selected to obtain a greater extent of collagen removal (28).

Specimens were then divided into 3 subgroups (n = 5) according to the adhesive and cement used: (1) self-adhesive cement RelyX U100; (2) two-step etch-and-rinse adhesive Single Bond 2 and dual-cured cement RelyX ARC; and (3) two-step self-etching adhesive Clearfil SE Bond and RelyX ARC. For the etch-and-rinse adhesive subgroup, the deproteinization process occurred after the acid etching.

All posts were coated with cement and slowly seated by finger pressure. Both adhesive systems and cements were applied following the manufacturers' instructions. They were continuously light-cured with a halogen light source at circa 500 mW/cm^2, as measured by the incorporated radiometer (Elipar Trilight; 3 M ESPE, Seefeld, Germany). Afterwards, specimens were stored in water at 37°C for 24 hours. All specimens were prepared under controlled humidity (55% $\pm$ 5%) and temperature (23°C $\pm$ 1°C) conditions.

After storage, each root was cut horizontally with a slow-speed, water-cooled diamond saw (Isomet 2000; Buehler Ltd, Lake Bluff, IL) to produce 1 slice approximately 1 mm thick for each root region (apical, middle, and cervical). As observed in a preliminary study, the cutting machine had to be set at 1.3-mm intervals to obtain 1-mm-thick slices because of the 0.3 mm attributed to the diamond disk thickness. Because the last 4 mm was sealed with endodontic material to simulate a clinical condition, typically 10 slices were obtained from each sample. The first slice was not included to avoid the influence of excess coronal material. Thus, the first 3 slices were considered as the coronal third, the next 3 slices the middle third, and the last 3 slices the apical third. One slice of each root region was selected for the

TABLE 2. Mean Bond Strength Values and Standard Deviations (Mpa) According to Treatments and Radicular Thirds

Material protocol	Radicular region	Treatment protocol	Mean (±SD)
Rely X U 100	Apical	Control	5.44 (1.36)^{cdefg}
		NaOCl	1.89 (1.61)^{abc}
		NaOCl + ascorbic acid	6.12 (1.72)^{cdefgh}
	Middle	Control	8.96 (1.54)^{fghij}
		NaOCl	3.87 (1.85)^{abcde}
		NaOCl + ascorbic acid	8.11 (1.09)^{efghij}
	Coronal	Control	10.10 (1.92)^{hij}
		NaOCl	4.53 (1.57)^{abcdef}
		NaOCl + ascorbic acid	12.26 (1.78)^{j}
Single Bond + Rely X ARC	Apical	Control	4.68 (1.77)^{abcdef}
		NaOCl	0.88 (0.60)^{ab}
		NaOCl + ascorbic acid	5.35 (2.90)^{bcdefg}
	Middle	Control	7.50 (2.80)^{defghi}
		NaOCl	1.86 (0.74)^{abc}
		NaOCl + ascorbic acid	8.07 (3.48)^{efghij}
	Coronal	Control	11.59 (3.03)^{ij}
		NaOCl	3.38 (1.47)^{abcd}
		NaOCl + ascorbic acid	10.16 (2.94)^{hij}
Clearfil SE Bond + Rely X ARC	Apical	Control	3.62 (0.67)^{abcde}
		NaOCl	0.80 (0.39)^{a}
		NaOCl + ascorbic acid	3.63 (1.26)^{abcde}
	Middle	Control	8.55 (1.12)^{fghij}
		NaOCl	3.70 (1.22)^{abcde}
		NaOCl + ascorbic acid	7.62 (1.54)^{defghi}
	Coronal	Control	11.91 (1.91)^{ij}
		NaOCl	5.31 (0.93)^{bcdefg}
		NaOCl + ascorbic acid	9.79 (2.53)^{ghij}

The same superscript letter indicates no statistically significant difference ($P < .05$).
SD, standard deviation.

push-out test. One operator prepared all slices, mixing them according to each third so that a second operator could select the slice in a random manner.

Slices of the restored roots were loaded in a universal testing machine (EMIC, São José dos Pinhais, Brazil) at a crosshead speed of 0.5 mm/min in the apical-coronal direction until post segment extrusion. The bond strength was calculated in MPa by dividing the load at failure (in N) by the area of the bonded interface. The area of the bonded interface was calculated as follows: $A = 2\pi rh$, where A is the area of the bonded interface, $\pi = 3.14$, r is the radius of post segment (mm), and h is the thickness of the post segment (mm).

Data were statistically analyzed by using a three-way analysis of variance and Dunnett T3 test ($\alpha = 0.05$).

Results

Mean values and standard deviations are presented in Table 2 and Fig. 1. Statistical analysis of data revealed significant differences between groups ($P < .05$) but no differences between cementing techniques ($P > .05$). An interaction effect between the surface treatment and root region was also observed ($P = .01$). Regardless of the material evaluated, the bond strength decreased when the root dentin was treated with 5% NaOCl ($P < .05$). Treatment with ascorbic acid was able to restore the bond strength to values similar to those in the control group. There were also significant differences among root regions, with bond strength varying in the following sequence: apical < middle < coronal. As analyzed by visual inspection with a 3× magnifying glass, all failures were primarily adhesive at the cement-dentin interface.

Discussion

The hypotheses studied were partially accepted. The irrigation protocols significantly influenced the shear bond strength, and there were regional differences between the radicular thirds. However, there were no differences between cementing techniques. The self-adhesive cement showed behavior similar to that of the other dual-cured cement, regardless of the irrigation protocol and adhesive system used.

Although they are relatively new, self-adhesive cements have shown promising laboratorial (23) and clinical (24) results. The main advantages advocated for this system are the ease of application and lower sensitivity to technique. As demonstrated in the present study, however, the application might still be sensitive to the irrigation protocol.

Debonding is still considered one important reason for the failure of intraradicular fiber posts (2). This type of failure is related to the degradation of exposed collagen that was incompletely covered by the adhesive (17). Therefore, it has been hypothesized that collagen removal with NaOCl might contribute to long-term adhesion stability (27, 30). Such treatment is recognized to remove the organic matrix, leaving a clean mineralized surface (27, 28). However, this effect is not always homogeneous. The concentration and application time play an important role in the process, with complete removal of collagen fibrils being achieved with application times exceeding 10 minutes (28).

When Single Bond 2 was evaluated in the present study, deproteinization occurred after phosphoric acid application. Although this procedure could potentially lead to a greater extent of collagen removal, no differences were found. It is possible that the deproteinizing process is less important than the redox effects of NaOCl and ascorbic acid to bond strength. Furthermore, the application of NaOCl after etching is opposite to the procedure used in clinical practice; this procedure has been evaluated in the literature as a possible method for minimizing the sensitivity of the hybridization technique (30, 31).

Many methods can measure the bond strength between endodontic posts and the tooth structure (25). In the present study, the push-out test was used because it has the benefit of more closely simulating clinical conditions. Shear stresses in this test occur at both the dentin/cement and post/cement interfaces (7), allowing evaluation

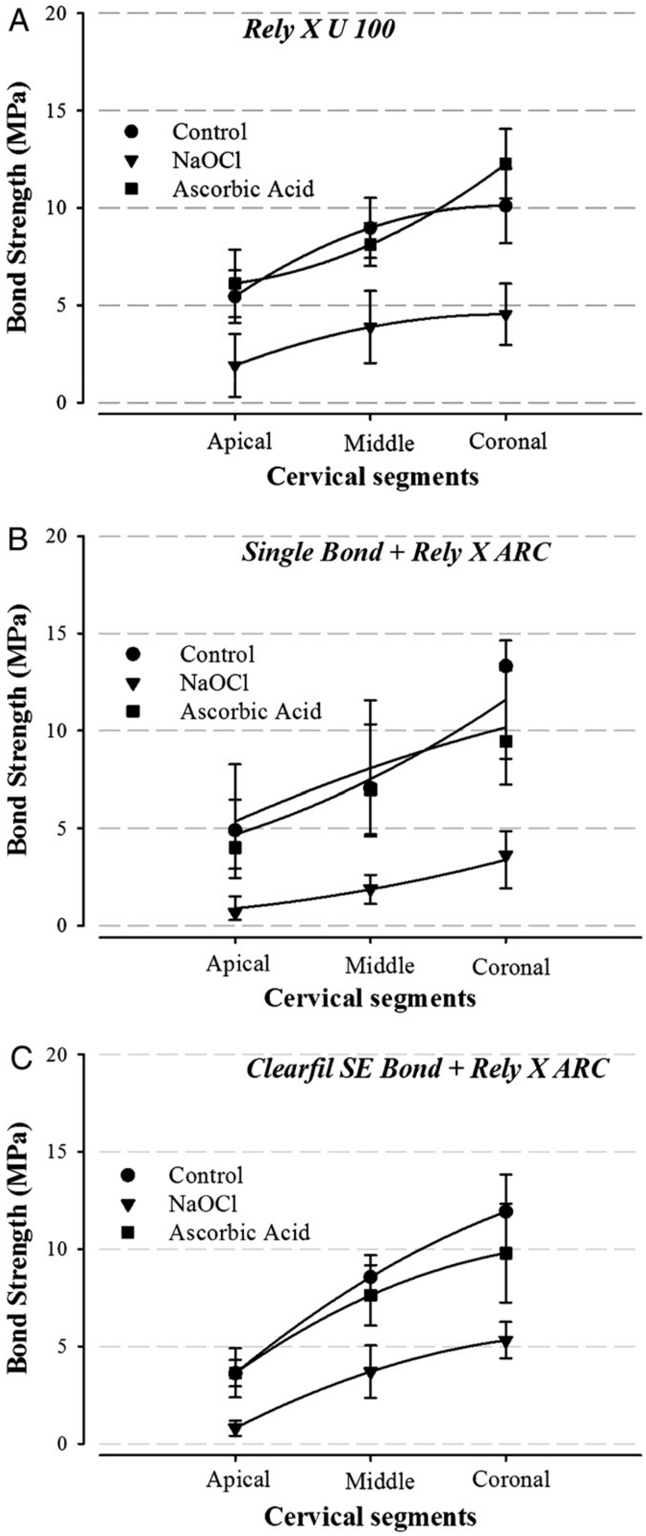

Figure 1. Mean bond strength values (MPa) according to the different treatments and radicular thirds for Rely X U100 (*A*), Single Bond + Rely X ARC (*B*), and Clearfil SE Bond + Rely X ARC (*C*).

even when bond strengths are low (15). However, the push-out test has the disadvantages of producing nonuniform stress at the adhesive interface (12), failing to measure bond strength on small surfaces, and requiring more teeth needed than the microtensile test (25).

Commercial brands of sealer and glass ionomer cement available in the Brazilian market were evaluated. It is known that eugenol-containing sealers might interfere with the adhesive properties of resin-based cements (6). However, Sealer 26 is a calcium hydroxide sealer. Furthermore, all materials (including sealer and gutta-percha) were removed from the lateral root canal walls during post space preparation. Moreover, Vidrion R was used only as a temporary restoration. Thus, these materials would not provide different results when compared with sealers and glass ionomer cements obtained from different manufacturers.

The NaOCl-treated group demonstrated significantly lower bond strength. The oxygen in NaOCl causes superficial oxidation and inhibits the interfacial polymerization of resin-based materials (33). The residual chemical solutions and their by-products likely diffuse into the dentin, affecting the polymerization of monomers at the demineralized dentin and decreasing the bond strength (13, 16). On the other hand, ascorbic acid was able to restore the bond strength of dentin after deproteinization with 5% NaOCl. These results are in agreement with those from previous studies (16, 32) and can be explained by the antioxidant ability of ascorbic acid. In addition, ascorbic acid and sodium ascorbate have also been used to reverse the oxidizing effect of bleaching agents (33). Ascorbic acid is nontoxic, and it is unlikely that its intraoral use will produce any adverse biologic effects (33).

The regional differences between the radicular thirds found are in agreement with findings from previous reports (20–22). These differences have been attributed to regional differences in the quantity, volume, and orientation of the tubules at different levels of the root canal (19). In addition, the access to light exposure and ease of access at the cervical level might play a role.

Scanning electron microscope (SEM) observations have been used to demonstrate the visual appearance of posts and dentin after push-out tests. Little additional information, however, is obtained from this analysis. Some studies demonstrated that SEM observations in situations similar to those in the present study are characterized mainly by adhesive failures, with a complete absence of cohesive fractures within the dentin or cement (9).

The clinician should be aware of the possible negative effects related to the use of NaOCl before adhesive techniques. Because the deproteinizing effect of NaOCl is a time-dependent process (28), it is important to notice that the results of the present study are applied to 10 minutes of continuous irrigation of both NaOCl and ascorbic acid. Although the irrigation protocols studied significantly influenced the bond strength, the time used is still excessive from a clinical point of view. For this reason, other application times should be considered along with the long-term bond strength stability of roots adhesively restored with fiber posts. In addition, both immediate and long-term NaOCl-deproteinized dentin bond strengths are dependent on the type of adhesive used (27). Therefore, the behavior observed in the present study might not be found when adhesives with different chemical properties are considered.

Conclusions

Deproteinization reduced bond strengths, whereas treatment with ascorbic acid reversed bond strengths to values similar to those of controls. Bond strength also varied according to the radicular region examined. RelyX U100 behaved similarly to RelyX ARC.

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